SUPPORTING INFORMATION

Nanoscale Protonation Limits and Charge Density in Polymer Films Govern the Activity of Immobilized LacZ under Acid Stress

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Supporting Methods: Circular dichroism spectroscopy

Freshly purified LacZ-WT and LacZ-ST were buffer-exchanged into 10 mM potassium phosphate buffers adjusted to pH 4–8 using a PD-10 desalting column (Cytiva, PD MidiTrap G-25, Cat. No. 28918008) and diluted to a final protein concentration of 2.5 mg mL^{-1}. CD spectra were recorded on a Jasco J-1500 spectropolarimeter using a quartz cuvette with a 0.1 cm path length over the wavelength range of 190–250 nm at room temperature. Each spectrum represents the average of three accumulations collected at a scanning speed of 50 nm min^{-1}, spectral bandwidth of 1 nm, data pitch of 0.1 nm, and D.I.T. of 8 s. Buffer blanks were measured and subtracted as background.

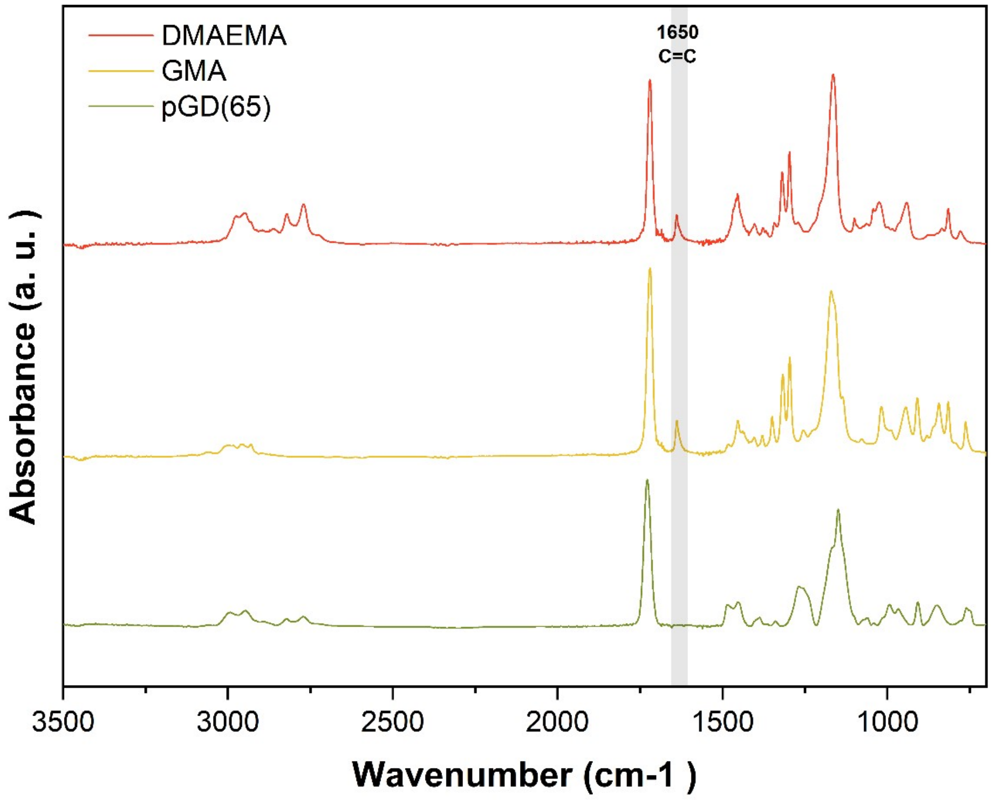

Figure S1. FTIR spectra of individual monomers (DMAEMA and GMA) and the resulting pGD(65) film. The characteristic vinyl (C=C) stretching vibrations observed in the monomers (1650 cm^{-1}) disappear in the polymer spectrum, confirming successful polymerization during the iCVD process.

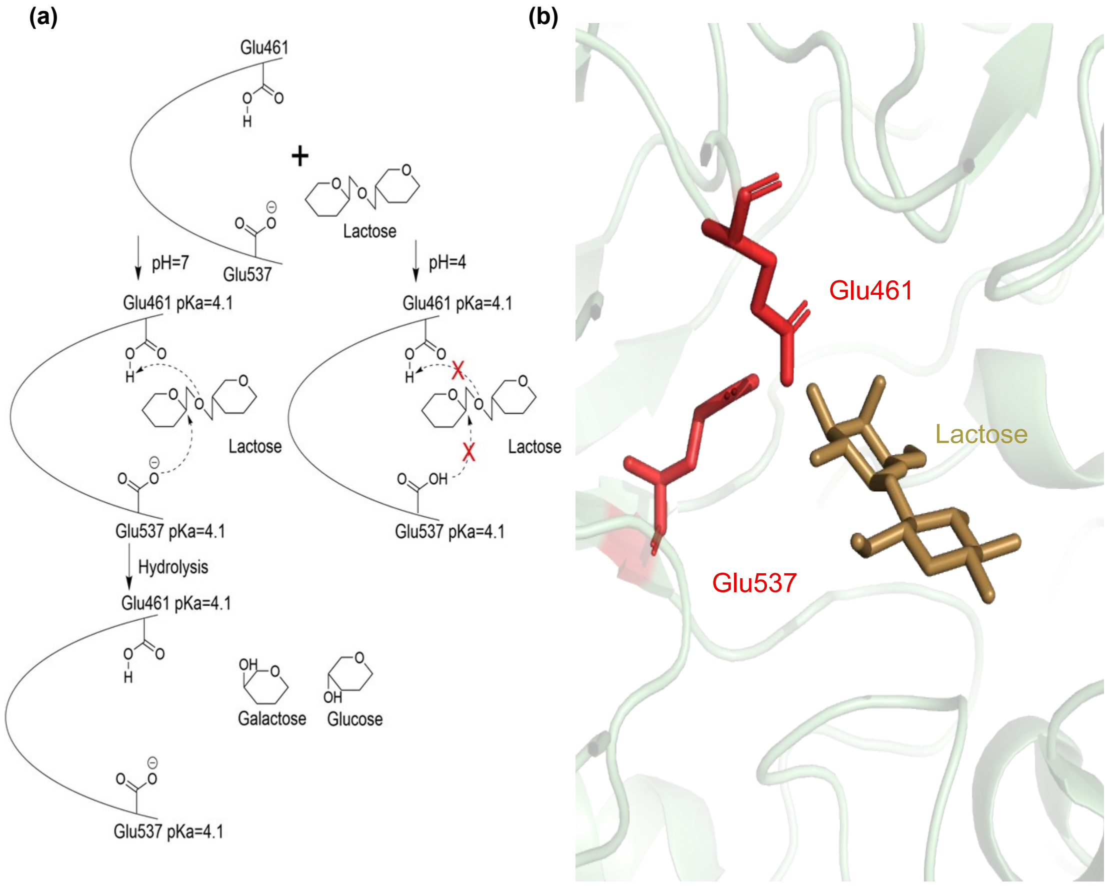

Figure S2. pH-dependent catalytic mechanism and active-site representation of β-galactosidase (LacZ protein). (a) LacZ protein catalyzes lactose hydrolysis through a double-displacement mechanism involving two key catalytic residues, Glu461 and Glu537. Under near-neutral conditions (pH =7), Glu537 remains deprotonated and acts as the nucleophile, attacking the glycosidic bond of lactose to form a covalent galactosyl–enzyme intermediate, while Glu461 functions as a general acid/base catalyst, facilitating proton transfer during bond cleavage and subsequent hydrolysis. This coordinated catalytic process results in the formation of the products galactose and glucose. In contrast, under acidic conditions (pH = 4), protonation of the glutamate residues (pKa ≈ 4.1) disrupts their catalytic roles. Protonated Glu537 loses nucleophilic reactivity, while protonation of Glu461 impairs its acid/base catalytic function, preventing effective cleavage of the glycosidic bond. As a result, lactose hydrolysis is inhibited under acidic conditions. (b) Structural representation of the LacZ active site showing key catalytic residues and substrate. Residues Glu461 and Glu537 are highlighted in red as stick models, and lactose is shown in gold. The protein backbone is displayed as a cartoon in light green. Labels indicate the positions of catalytic residues and substrate within the active site.

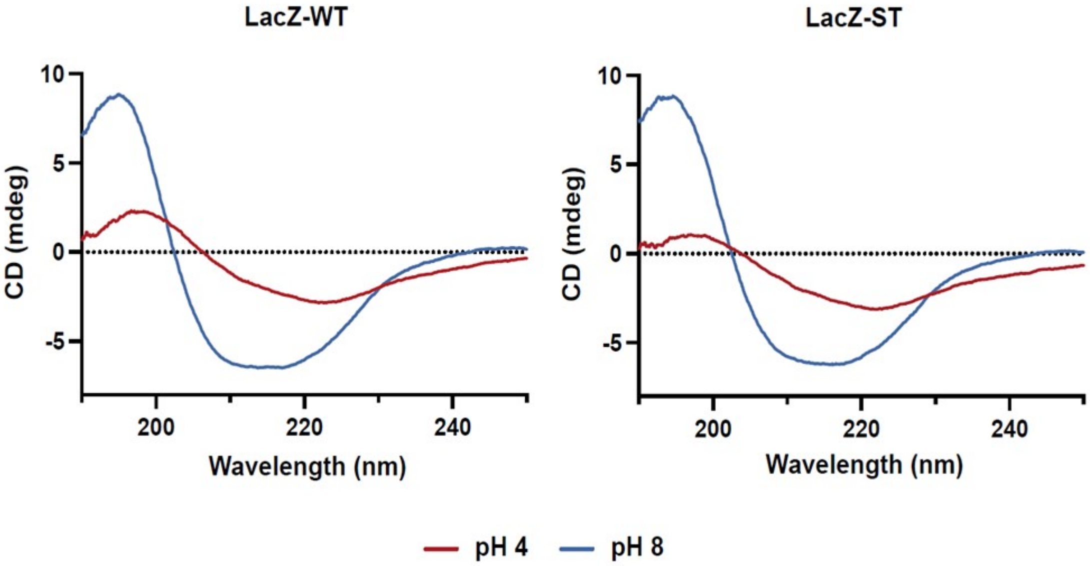

Figure S3. Circular dichroism (CD) spectra of LacZ-WT and LacZ-ST at pH 4 and 8, recorded in the wavelength region (190–250 nm). Spectral features characteristic of α-helical structures, including a positive band near 195 nm and negative minima at ~208 and ~222 nm, are observed at pH 6–8, while reduced ellipticity is evident at pH 4.

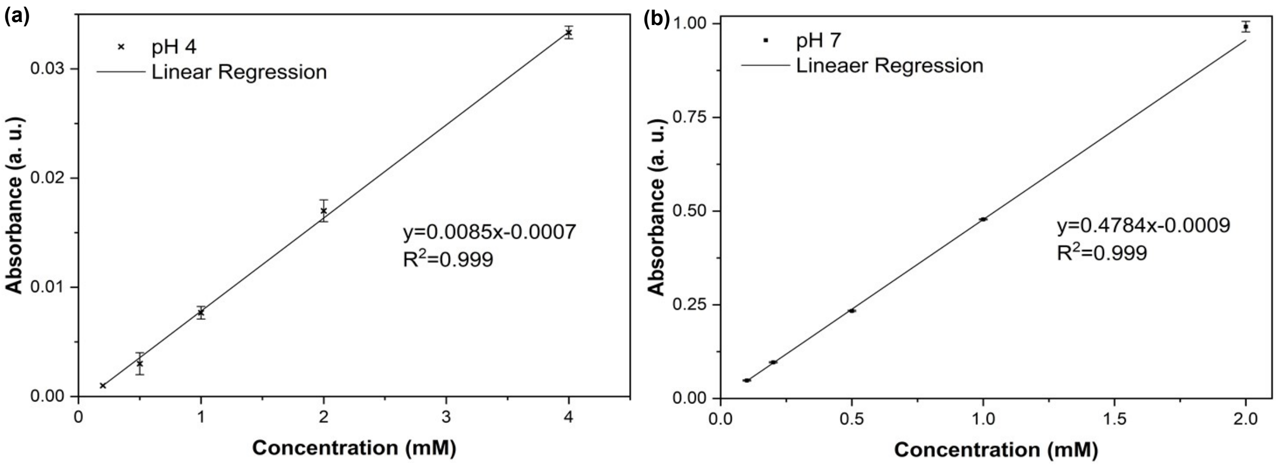

Figure S4. Calibration curves for ONP absorbance as a function of concentration under different pH conditions. Linear regressions were performed at (a) pH 4 and (b) pH7, yielding excellent linearity (R^2 = 0.999) in both cases

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Table S1. Initiated chemical vapor deposition (iCVD) process parameters for synthesizing the enzyme immobilization supports.

Samples	Flow rates (sccm)				
	(Co)monomers		TBPO	Argon	Total
	G	D			
pGD(25)	2.0	0.6	0.6	0.0	3.2
pGD(65)	1.0	1.0	0.6	0.4	3.0

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Table S2. Pressure saturation ratios (Pm/Psat) of (co)monomer gases and the resulted deposition rates of polymer thin films

Sample	P_m/P_{sat} (on iCVD stage)			Deposition rates (nm min^{-1})*		
	G	D	Total	DR_{stg}	DR_{plt}	DR_{stg}/DR_{plt}
pGD(25)	0.07	0.07	0.14	96.5	5.7	16.8
pGD(65)	0.04	0.13	0.17	46.1	0.5	94.1

*DR_{stg} and DR_{plt} represent the deposition rates measured on a Si substrate placed on the iCVD stage and those measured on another Si substrate located atop a 96-well plate, respectively. The 96-well plates were placed on custom-made aluminum plates that fit the shape of the plates well in order to facilitate heat transfer to the iCVD stage.

Table S3. Copolymer molar compositions determined based on transmission FTIR.

Samples	Copolymer composition (mol%)	
	G	D
pGD(25)	76	24
pGD(65)	35	65

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Table S4. Primer information

Primer	Sequences (5' to 3')
LacZ-WT-Nde I-F	aagaaggagatatacatatgaccatgattacggattcactggc
LacZ-LacZ-WT-6xHis-R	caaaacagccaagcttttagtgatggtgatggtgatgtttttgacaccagaccaact
SpyTag-GSlinker-F	tttaagaaggagatatacatatggcgcatatcgtgatggtggatgc
SpyTag-GSlinker-R	ttccggatccacctccggatcctttggtcggtttatacgcatccaccatcacgatatg
pBAD-LacZ-WT-spy-F	gaggtggatccggaagtggtggatccggaagtggatccggaagtatgaccatgattacgg
pBAD-lacz-wt-spy-r	gtatatctccttcttaaagttaaacaaaattatttctagcccaaaaaacgggtatgg
SpyCatcher-Nde I-F	aagaaggagatatacatatggttgataccttatcaggtttatcaagtgagc
SpyCatcher-Hind III-6xHis-R	caaaacagccaagcttttagtgatggtgatggtgatgtaaatatgagcgtcacctttag

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Gene Blocks information

SpyCatcher:

5’atggttgataccttatcaggtttatcaagtgagcaaggtcagtccggtgatatgacaattgaagaagatagt
gctacccatattaaattctcaaaacgtgatgaggacggcaaagagttagctggtgcaactatggagttgcgt
gattcatctggtaaaactattagtacatggatttcagatggacaagtgaaagatttctacctgtatccaggaaa
atatacatttgtcgaaaccgcagcaccagacggttatgaggtagcaactgctattacctttacagttaatgag
caaggtcaggttactgtaaatggcaaagcaactaaaggtgacgctcatattta3’

Table S5. Thickness of pGD(65) films before and after soaking for 5 mins in 0.1 mM HCl solution (pH 4), demonstrating the stability of the iCVD polymer coatings.

Immobilization Support	Thickness (nm)	Thickness after soaking (nm)
pGD(65) 50nm	48.3 ± 0.8	48.5 ± 2.8
pGD(65) 100nm	99.7 ± 1.9	93.9 ± 5.8
pGD(65) 200nm	179.1 ± 9.3	184.5 ± 16.1
pGD(65) 400nm	436.8 ± 18.3	430.9 ± 28.3
pGD(65) 800nm	768.9 ± 16.0	753.1 ± 17.7

Table S6. Quantitative FTIR peak areas of C=O and N–H stretching bands and their normalized ratios (N–H/C=O) for pGD(65) films with varying thickness after immersion in acidic solution (pH 4, 0.1 mM HCl) for 5 min followed by air-drying for 1 min.

Polymer	Area(C=O)	Area(N-H)	Ratio (N-H/C=O)	Normalized Ratio (against the 100 nm)
pGD(65) 100nm	0.984	1.888	1.918	1.000
pGD(65) 200nm	1.666	3.320	1.992	1.038
pGD(65) 300nm	2.348	3.780	1.610	0.839
pGD(65) 400nm	2.546	3.224	1.266	0.660

Table S7. Random Immobilization (RI) efficiency for various immobilization supports used in this study. Values tabulated below are mean ± standard error (N = 3).

Immobilization Support	No. LacZ-WT /100nm^2 *	immobilization yield (IY) (%)
RI-SGD(65)	41.1 ± 1.9	9.3 ± 0.4[a]
RI-pGD(65) 50nm	41.7 ± 2.0	9.4 ± 0.5[a]
RI-pGD(65) 100nm	35.1 ± 1.6	7.9 ± 0.4[b]
RI-pGD(65) 200nm	34.0 ± 3.0	7.7 ± 0.7[b]
RI-pGD(65) 400nm	42.6 ± 2.7	9.6 ± 0.6[a]
RI-pGD(65) 800nm	35.8 ± 3.4	8.1 ± 0.8[b]

* LacZ-WT in tetramer form was used for calculating the population density on surface

Table S8. Directed Immobilization (DI) efficiency for various immobilization supports used in this study. Values tabulated below are mean ± standard error (N = 3).

Immobilization Support	No. LacZ-ST /100nm^2 *	SC occupied (%) **	immobilization yield (IY) (%)
DI-SGD(65)	6.3 ± 1.1^{ab}	11.3 ± 1.9^{a}	1.7 ± 0.3^{a}
DI-pGD(65) 50nm	4.7 ± 0.7^{bc}	8.8 ± 1.4^{ab}	1.3 ± 0.2^{b}
DI-pGD(65) 100nm	3.8 ± 0.4^{c}	10.3 ± 1.0^{a}	1.0 ± 0.1^{b}
DI-pGD(65) 200nm	7.5 ± 0.8^{a}	16.7 ± 1.7^{c}	2.0 ± 0.2^{c}
DI-pGD(65) 400nm	5.0 ± 1.1^{bc}	11.0 ± 2.5^{a}	1.4 ± 0.3^{ab}
DI-pGD(65) 800nm	7.6 ± 1.1^{a}	6.1 ± 0.9^{b}	2.1 ± 0.3^{c}

* LacZ-ST in tetramer form was used for calculating the population density on the 96 well plate.

** "SC occupied (%)" is the percentage of SpyCatcher occupied by LacZ-ST teramers, computed using the following formula:

$$SC\ occupied\ (\%) = \frac{No.\,LacZ - ST(teramers)}{No.\ SC} \times 10$$